



# *Environmental Effects of Dredging Technical Notes*



## WETLAND ANIMAL BIOASSAY OF SALTWATER DREDGED MATERIAL

**PURPOSE:** This note introduces the concept of using a wetland animal as an indicator of the contaminants in dredged material proposed for disposal in a wetland environment. An example of the application of an animal bioassay procedure to saltwater dredged material in a wetland creation environment was reported in a paper entitled "Application of a Wetland Animal Bioassay for Determining Toxic Metal Uptake from Dredged Material," which was presented at the International Symposium of Ecotoxicological Testing for the Marine Environment (Simmers, Rhett, and Lee 1984). The text of this note was taken from the paper.

**BACKGROUND:** Animal bioassay test procedures are being evaluated and field tested and verified under the "Interagency Field Verification of Testing and Predictive Methodologies for Dredged Material Disposal Alternatives," called the Field Verification Program (FVP). The FVP research is being conducted in conjunction with a scheduled dredging project in Black Rock Harbor near Bridgeport, Connecticut. The procedures are relatively simple and can provide information that may be required in the ecological evaluation and environmental assessment of dredged material disposal. Based on laboratory results and limited field testing, the procedures can be applied to saltwater sediment (dredged material) that requires placement in a wetland environment. The concept presented in this note is the result of ongoing research under the FVP. Draft final guidance will be completed in September 1987.

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### Introduction

The Clean Water Act requires that the environmental evaluation of dredged material prior to discharge or impacting the waters of the United States include the effects of disposal on contaminant concentrations through biological processes. This resulted in a need for Corps of Engineers

Districts to be able to predict the potential contamination of animals that may be associated with each of these potential disposal alternatives: open-water disposal, upland disposal, and/or wetland creation. The following is a summary of a wetland animal solid-phase bioassay test applied to sediment collected from the waterway at Black Rock Harbor (BRH), Bridgeport, Connecticut. This test procedure was designed to evaluate the potential movement of toxic heavy metals and other contaminants from dredged material placed in a wetland (reduced) environment into sediment-dwelling intertidal invertebrates as a first step that may be used to evaluate contaminant mobility to animals that may colonize the dredged material. No inference on the movement of contaminants through the wetland food web is offered at this time.

### Methodology

The sandworm *Nereis virens* was selected as the test animal. A stock of the worms was obtained from supplier in Maine and held in tanks of 22 ppt salinity seawater (Instant Ocean) with a sand substrate.

The contaminated sediment used in the tests was collected from an area of BRH that was scheduled to be dredged. Sediment near the mouth of the harbor (an area of lesser contamination) was collected for use as a reference material. Both types of sediment were kept flooded (i.e., in a reduced condition) while in storage and throughout the test procedure. The sand used as a third test medium was the same used in the holding tanks. Results of bioassay tests of animals from aquaria with the sand substrate provided information on background levels of the contaminants of interest.

Preliminary screening tests were used to determine if dilution of the contaminated sediment would be necessary to ensure the survival of all test animals for the maximum exposure period of 14 days. The same sand as that used in the holding-tank was used as a dilution medium. Based on the results of the screening tests, a mixture of 75 percent sand and 25 percent BRH sediment was selected for use as the contaminated sediment substrate. The reference control sediment or the sand was used as the substrate in the other aquaria.

Twelve sandworms (26 g total wet weight) were placed in 3.2 L of each substrate in aquaria. The medium was kept under 15 cm of seawater and aerated. All of the aquaria were placed in a constant temperature bath at 17° C.

At the end of the 7- and 14-day test periods, the worms were harvested, counted, weighed, and allowed to depurate in clean sand for 24 hr. The worms were then killed in boiling water and homogenized in a stainless steel Sorvall omni-mixer (DuPont Co., Newton, CT 06470). A portion of the homogenized tissue was frozen for future needs, and the remainder was oven-dried prior to analysis for arsenic, cadmium, chromium, copper, lead, mercury, nickel, and zinc.

### Results

The preliminary screening tests indicated that the BRH sediment was toxic to the sandworms. This toxicity did not occur in the reference sediment. The screening tests indicated that survival for up to 14 days could be obtained if the sediment was diluted with 75 percent sand. Sand of the same kind used in the maintenance cultures of the test animals was used as a dilution medium.

The weights and number of worms surviving in the three treatment groups at 14 days is shown in the following tabulation. There was some weight loss

Substrate	Numbers ( $\bar{X}$ ) and Mean Weight of Worms, g (wet wt)		14-day Weight Loss, %
	Initial	Final	
Sand	$\bar{X} = 10.5$ $27.07 \pm 0.3$	$\bar{X} = 10.25$ $21.84 \pm 2.4$	19
75% sand/25% BRH sediment	$\bar{X} = 12.5$ $26.86 \pm 0.8$	$\bar{X} = 12$ $21.51 \pm 2.0$	20
Reference sediment	$\bar{X} = 11.5$ $26.71 \pm 0.7$	$\bar{X} = 9.75$ $19.17 \pm 2.4$	27

Note: Data represents composite of four replications.

in all three groups and a slight decrease in worm numbers. Since none of the worms in any treatment group were fed, the weight loss may have been due to starvation: the worms in the sand medium lost about the same weight as those in the reference and contaminated sediments. Based on the weight comparisons, the worms in the contaminated sediment did not appear to be stressed more than those in the reference sediment or the sand.

The tests using the sand substrate differed from the maintenance culture only in that the worms were not fed. This treatment represented the background levels after 7 and 14 days depuration in clean sand (Table 1). These data indicate the levels of heavy metals that would be retained in worms

Table 1  
Contaminant Concentrations in Substrate and Animal Tissue

Substrate	Contaminant	Contaminant Concentration, $\mu\text{g/g}$ (dry wt)*		
		Substrate	Tissue	
			7 Days	14 Days
Sand	Arsenic	<0.49	$0.80 \pm 0.13$	<1.20
	Cadmium	0.1	$0.63 \pm 0.05$	$0.20 \pm 0.04$
	Chromium	0.9	$3.15 \pm 0.34$	$4.56 \pm 1.10$
	Copper	<4.9	$14.1 \pm 2.6$	$17.9 \pm 1.5$
	Lead	13.3	$2.95 \pm 0.45$	$5.47 \pm 1.17$
	Nickel	0.2	$4.15 \pm 1.75$	$11.22 \pm 4.18$
	Zinc	<4.9	$193 \pm 58$	$176.2 \pm 16$
75% sand/25% BRH sediment	Arsenic	0.47	$1.40 \pm 0.42$	$1.07 \pm 0.13$
	Cadmium	0.4	$1.08 \pm 0.35$	$2.95 \pm 0.26$
	Chromium	92.3	$3.15 \pm 1.42$	$10.96 \pm 3.51$
	Copper	153.0	$13.9 \pm 1.4$	$168.0 \pm 28$
	Lead	22.4	$3.06 \pm 0.30$	$5.67 \pm 3.17$
	Nickel	12.9	$9.32 \pm 1.70$	$11.55 \pm 6.69$
	Zinc	83.6	$270.6 \pm 95.6$	$206.4 \pm 40.3$
Reference sediment	Arsenic	4.9	$0.64 \pm 0.17$	1.20
	Cadmium	0.3	$0.48 \pm 0.12$	$1.42 \pm 0.10$
	Chromium	230.5	$6.98 \pm 4.51$	$4.42 \pm 2.39$
	Copper	326.5	$13.4 \pm 1.3$	$28.1 \pm 6.2$
	Lead	75.2	$2.95 \pm 0.17$	$3.06 \pm 1.15$
	Nickel	36.2	$3.07 \pm 0.65$	$6.33 \pm 1.28$
	Zinc	256.5	$190 \pm 38$	$353.2 \pm 163.8$

\* Each value is mean of 4 replicates.

All mercury concentrations at or below detection limit ( $0.1 \mu\text{g/g}$ ).

caught in an area of naturally occurring sediment and maintained for 30 days in clean sand prior to the depuration and analysis. The analyses of the worms in the BRH sediment/sand mixture and the reference medium are shown in Table 1.

In all the test media and tissue samples, mercury concentrations were near or below detection limits of  $0.1 \mu\text{g/g}$ . The sediment concentrations varied for arsenic, chromium, lead, and zinc, although the animal tissue levels remained much the same. Cadmium remained relatively constant with the exception of the 14-day tissue samples from the BRH sediment/sand medium. The observed levels of nickel were quite variable in both the sediment and animal tissue samples. Only copper showed a marked increase in animal tissues exposed to the diluted BRH sediment test medium.

## Discussion

The apparent levels of arsenic, cadmium, copper, and zinc are above sediment levels and deserve some note. Arsenic accumulation by *Nereis* has been investigated by Bryan and Gibbs (1983). From exposure to sediment containing 2520  $\mu\text{g/g}$ , these authors found 87  $\mu\text{g/g}$  in the tissues of *N. diversicolor* in comparison to 7  $\mu\text{g/g}$  tissue arsenic in normal sediments containing 13  $\mu\text{g/g}$  arsenic. The values appearing in Table 1 indicate that the arsenic levels both in the diluted BRH sediment and in the animals exposed to it are very low in comparison to those of Bryan and Gibbs (1983). The very low sediment arsenic content initially would explain the insignificant uptake by the sandworms.

Due to ecotoxicological significance of cadmium, any indication of accumulation of this metal is highly significant. Although the movement of cadmium to *Nereis* appears to be through the interstitial water shown by Ray et al. (1980), the availability of the cadmium from the sediment is of concern.

A review of the literature indicates that the tissue levels of cadmium after exposure to contaminated sediments as shown in Table 1 are generally higher than other reported values. Luoma and Bryan (1982) found levels of 0.20-3.10  $\mu\text{g/g}$  in *N. diversicolor* from sediments containing 0.1-10.8  $\mu\text{g/g}$  cadmium. Bryan and Hummerstone (1973) found cadmium levels in *N. diversicolor* of 0.12-0.56  $\mu\text{g/g}$  (dry weight) from sediments with cadmium levels similar to that of the diluted BRH sediment. This may indicate that the cadmium in the BRH material is more available to *N. virens* and/or may represent species differences within *Nereis*.

Copper appeared to be accumulated in the bioassay animals placed in the diluted BRH sediment (Table 1). Bryan and Hummerstone (1973) found that in sediment containing 17-3052  $\mu\text{g/g}$  of copper, the copper content of *N. diversicolor* did not exceed 68 percent of the sediment concentration. This suggests that the copper in the diluted BRH material is quite available and is taken up by *Nereis*.

The accumulation of zinc in *Nereis* is relatively uniform across the three sediments. Bryan (1976) indicated that in *N. diversicolor*, zinc is actively accumulated and controlled as a metabolite. The zinc levels found in these bioassay organisms tend to substantiate the concept of physiological

regulation of zinc by *Nereis* and suggest that at the sediment concentrations studied, zinc is not of concern to *Nereis*.

### Conclusions

The sandworm screening test demonstrated that the BRH sediment is toxic to intertidal annelids when maintained in a reduced condition. The bioassay showed that of the heavy metals present in the sediment, only copper and, to a lesser extent, cadmium are bioaccumulated. If the BRH sediment were mixed with uncontaminated sediment and used to create a wetland, there is a possibility of copper and cadmium accumulation in *N. virens*, and, as Ray et al. (1980) suggested, cadmium may accumulate to elevated levels in *Nereis* living full 5- to 6-year life spans. The comparison of the levels of metals in the three test substrates (Table 1) suggests that the toxicity observed in the initial screening portion of the test was not due to the uptake of toxic heavy metals but to something else.

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